ELECTRON MICROSCOPIC STUDIES ON FAT METABOLISM IN THE LIVER

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by

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I. INTRODUCTION

Previously, Dr. NAKAMURA in our research labolatory carried out electron microscopic studies on the intrahepatic metabolic process of intravenously administered sesame oil emulsion which mainly contained triglyceride and arrived at the following conclusion. The intravenously infused glyceride is first taken into the sinusoidal endothelial cell and then finally enters into the cytoplasm of the liver parenchymal cell through DISSE's space by the blood stream in the form of α-lipoprotein, after being transformed into phospholipid by the action of mitochondria.

On the other hand, as already known, the orally administered fat is digested and absorbed from the intestinal canal, and then transfered into the thoracic chyle. Accordingly, the author attempted the following experiments for the purpose of studying the intrahepatic metabolic process of the physiologically digested and absorbed fat. That is, infusing the thoracic chyle, being collected from the thoracic duct of a cat forced on fat, into the vein of the same cat after a definite interval, the author studied electron microscopically the intrahepatic metabolic process of the chylomicra contained in the thoracic chyle.

II. MATERIALS AND METHODS

(A) Materials

1) Fat by Oral Administration: To obtain thoracic chyle containing fat of high concentration, fat should be prepared in an easily digestible and absorbable form prior to administration. Therefore, the triglyceride emulsion containing myristic, palmitic, stearic, oleic, linoleic and linolenic acids was made in our laboratory, and was used in this experiment as fat for oral administration.

2) Experimental Animals: Adult cats representing carnivores whose fat absorption and fat disposal are vigorous and each weighing approximately 3～4 kg, were used.

(B) Methods

1) Methods of Collecting Chyle: The adult cats in the post-absorptive state received the above-mentioned triglyceride emulsion by an orally inserted stomach tube so that 4 g of fat could be administered per kg of body weight. One and one-half hour after oral administration, thoracic chyle was collected from the thoracic duct by the indirect method shown in Fig. 1. Namely, a 4 cm long skin incision was made in the left supraclavicular fossa and carried down through subcutaneous adipose tissue and fascia in the same direction

and length. Thus, the left subclavian, left jugular, and left brachiocephalic vein were exposed with utmost care not to damage the apical pleura. As the thoracic duct terminates into the branching portion of the left subclavian vein from the left internal jugular vein, the internal jugular, the left subclavian and the left brachiocephalic vein were ligated completely and closure of the lumen opening into the thoracic duct was made. At the same time, several small veins emptying into above-mentioned veins must be ligated completely lest the thoracic chyle should flow into the venous blood stream. Then, a polyethylene tube of 3 mm in diameter was inserted into the left internal jugular vein proximal to the ligated point and thoracic chyle was collected in the sterilized small flasks. Furthermore, to avoid bacterial contamination, the cats were put on 100,000 units of penicillin injected subcutaneously before operation, and about 1,000 units of crystalline penicillin per 10 cc of thoracic chyle were added to the collecting flasks. Collecting flasks filled with thoracic chyle were immediately placed in the refrigerator, and thoracic chyle never coagulated without addition of heparin, if filtered gently with gauze before use. It took about two to three hours to collect the needed amount of chyle. The cats were kept free except during the operating and collecting periods, so as to enable them to return to the normal state as soon as possible.

2) Fat Components of Chyle: The average amount of each fat component in thoracic chyle following oral administration of the triglyceride emulsion is shown in Table 1. The quantitative determination of each fat component was made by the following methods, the method of VAN de KAMER A and B for the tatal fatty acid and glyceride, the method of BLOOR for phospholipid and the method of RAPPAPORT for cholesterol.

Table 1. Fat Components in Cat Chyle Following Oral Administration of Triglyceride Emulsion

Total lipids	Glyceride	Phospholipid	Cholesterol
5847mg/dl	4465mg/dl	873mg/dl	509mg/dl
100%	76.4%	14.9%	8.7%

3) Method of Injection and Dose of Chyle: A definite quantity of the collected chyle was injected intravenously into the femoral vein of the same cats from which the chyle had been collected. The dose injected was equivalent to 0.5 g of fat per kg of body weight.

4) Method of Making Electron Microscopic Preparations: The cats were laparotomized under no anesthesia successively 10, 30 minutes and 1, 2, 3, and 6 hours after the intravenous administration of thoracic chyle, and then specimens of a certain size were excised from the central portion of the right lobe of the liver. These specimens were immediately cut to pieces about 1～1.5 mm^3 in size, put in a M/25 isotonic sucrose solution containing 1% osmic acid which was maintained at pH 7.2 with a veronal buffer solution, and fixed for 2 hours in a refrigerator. For dehydration of the tissue, a series of alcohol solutions was employed (The tissue was put for 10 minutes each in 30%, 40%, 50%, 60%, 70%, 80%, 90% and 95% alcohol and twice for 30 minutes in 100% alcohol.). Then, the dehydrated tissue was immersed in a mixed solution of n-butyl- and methyl-methacrilate at a ratio of 6 : 4, and kept in a refrigerator for about 24 hours.

The solution was then packed into a No. 0 capsule, and the tissue was embedded in it and heated at 55°C in an incubator for polymerization.

A SHIMADZU ultramicrotome was used in preparing the ultra thin sections, and observation was done with an AKASHI Tronscope TRS-50-E type and the HITACHI Electron microscope HS-6.

II. OBSERVATIONS

Morphological changes of the sinusoidal lumen, sinusoidal endothelial cells and hepatic parenchymal cells at 10, 30 minutes and 1, 2, 3, and 6 hours after the intravenous administration of thoracic chyle were observed under the electron microscope and the following results were obtained:

(1) 10 Minutes after the Intravenous Administration of Thoracic Chyle

1) Sinusoidal Lumen and Sinusoidal Endothelial Cells (Plate 1)

A number of chylomicra (0.15～0.5 μ in diameter) are observed in the sinusoidal lumen as touching the plasma membrane, and pseudopods of the sinusoidal endothelial cells. Findings, however, suggesting thrombosis or embolism of the sinusoidal lumen with chylomicra are never seen. Being surrounded with a common encircling membrane which is formed by vesiculation of the plasma membrane, several chylomicra are taken into the sinusoidal endothelial cell. Sometimes, scores of chylomicra are surrounded altogether with an encircling membrane, therefore, diameters of the encircling membranes are in a wide range from 0.5 to 5 μ. Chylomicra within the encircling membranes are sharply defined from others and present the same globular form as that of chylomicra in the sinusoidal lumen (Plate 2). The double membranous structure of the encircling membrane is clearly observed under 20,000～50,000 magnifications. The cytoplasm of the sinusoidal endothelial cell is opaque, presents the same electron density as that of the hepatic parenchymal cells, and shows a fine granular structure. Mitochondria of the sinusoidal endothelial cell are much smaller and fewer than that of the hepatic parenchymal cell. Phagocytized erythrocytes are often found in the sinusoidal endothelial cell. As already mentioned by NAKAMURA, the fact that the cytoplasm of the sinusoidal endothelial cell was elongated at both ends and formed thin sinusoidal endothelium which possessed small pores here and there like a mesh, is also recognized in this experiment. Furthermore, this hepatic sinusoidal endothelium is devoid of basement membrane, being different from the other capillary endothelium, and is directly in contact with microvilli protruding from the hepatic parenchymal cell. Between the sinusoidal endothelium and microvilli, so-called "DISSE's space" is observed. Accordingly, it is considered that the hepatic parenchymal cell is directly in contact with the blood stream through DISSE's space and pores of the sinusoidal endothelium (Plate 3).

2) Hepatic Parenchymal Cell

At the time of observation, many globular fat droplets of about 1 μ in diameter are recongnized in the hepatic parenchymal cell and mitochondria come to gather around these fat droplets at their lateral surface (Plate 4). Still more, it is observed under high magnification that the outer layer of mitochondria facing on the fat droplets becomes obscure or completely disappears and mitochondrial crests are arranged at right angles

to the contact surface (Plate 5). There are 2 or 3 microbodies in the hepatic parenchymal cell in this stage and they are globular in form, 0.1～0.5 μ in diameter and are limited by a single, well defined membrane, and their matrix contains a finely granulated substance that may condense in the center, forming an opaque and homogenous core (Plate 6).

(2) 30 Minutes after the Intravenous Administration of Thoracic Chyle

1) Sinusoidal Lumen and Sinusoidal Endothelial Cell (Plate 7)

In the sinusoidal lumen, chylomicra are slightly recognizable already and the surface of the sinusoidal endothelium is flattened and devoid of pseudopods. Outlines of chylomicra within the encircling membranes become obscure and coalesced into larger homogenous fat droplets, and at the same time, the encircling membranes disappear. Mitochondria of the sinusoidal endothelial cell increase in number and have a tendency to gather around the larger fat droplets which are formed by coalescence of chylomicra. Electron density of the mitochondrial crests is increased and the crests are well defined. Marked changes are not observed in DISSE's space in this stage.

2) Hepatic Parenchymal Cell (Plate 8)

Being different from the case of 10 minutes after injection, fat droplets are seldom found in the hepatic parenchymal cell. Mitochondrial crests become very distinct, much increased in number, and the direction of their arrangement are varied and not constant. Large mitochondria possessing hundreds of crests are found to and fro (Plate 9). Microbodies increased also in number and in size. About 5 or 6 microbodies (0.5～1.0 μ in diameter) are usually seen in a hepatic parenchymal cell. Their matrix is homogenous, finely granulated and several granules of high electron density are observed near its center. Furthermore, a fine concentric stratified structure is discovered in the peripheral zone of the microbody (Plate 10).

(3) One Hour after the Intravenous Administration of Thoracic Chyle

1) Sinusoidal Lumen and Sinusoidal Endothelial Cell (Plate 11)

Except for a few erythrocytes, nothing is seen in the sinusoidal lumen. The sinusoidal endothelial cell showed a form as if it were elongated along the lumen. Chylomicra taken into the sinusoidal endothelial cell by vesiculation of the plasma membrane have coalesced into larger droplets within the encircling membranes in this stage, and the encircling membranes have vanished at the same time. The mitochondrial outer layer contacting with these droplets also vanishes and mitochondrial crests are placed at right angles to the contact surface. On the other hand, in the protoplasm facing on DISSE's space lipid particles of about 300 Å in diameter begin to appear, and then these lipid particles, several as a group, are transfered to DISSE's space being encircled by a kind of membrane. But it is completely unknown from what origin the membrane surrounding these lipid particles develops. Thus, in DISSE's space, many lipid particles of about 300 Å in diameter are observed (Plate 12).

2) Hepatic Parenchymal Cell (Plate 13)

Lipid particles of about 300 Å in diameter which have appeared in DISSE's space are surrounded, 2 or 3 particles as a group, by the encircling membrane originating from the surface of the microvilli and are transfered into the hepatic parenchymal cell. Mito-

chondria and the nuclei have a tendency to shift to the peripheral zone of the hepatic parenchymal cell facing on DISSE's space. Microbodies are observed in the central zone of the cell and the change in their number is not remarkable in comparison with the case of 30 minutes after injection, but as a whole, their electron density seems to be increased.

(4) Two Hours after the Intravenous Administration of Thoracic Chyle

1) Sinusoidal Lumen and Sinusoidal Endothelial Cell (Plate 14)

There are no changes worth noting in the sinusoidal lumen at all. The surface of the sinusoidal endothelial cell is smooth and totally devoid of pseudopods. Chylomicra are scarcely observed in the sinusoidal endothelial cell and here and there a few phagocytized erythrocytes and ferritin granules which are the decomposed substance of erythrocytes, are also observable. Mitochondria decrease in number and only about 3 or 4 are observed in a cell, and at the same time, mitochondrial crests diminish in number, too. Sometimes, almost structureless mitochondria with a homogenous matrix are found in this stage. In DISSE's space, lipid particles are scarcely observed.

2) Hepatic Parenchymal Cell (Plate 15)

As a marked change, many globular fat droplets of about 1 μ in diameter appear in the cytoplasm of the hepatic parenchymal cell. Of course, adhering to the surface of fat droplets or around them, lipid particles taken from DISSE's space by vesiculation of microvilli are observed contemporaneously in this stage. In other words, it is considered that lipid particles of 300 Å in diameter gradually coalesce into fat droplets of about 1 μ in diameter, after they have been transported into the hepatic parenchymal cell and have lost their encircling membranes. Mitochondria come together around these fat droplets. Microbodies increase prominently in number and in size, and become as large as mitochondria, or sometimes are far larger than mitochondria. Some of these microbodies exhibit an intermediate form between the microbody and mitochondria, that is, a mitochondrial crest-like structure is found in the matrix.

(5) Three Hours after the Intravenous Administration of Thoracic Chyle

1) Sinusoidal Lumen and Sinusoidal Endothelial Cell (Plate 16)

The sinusoidal lumen is vacant as before. The plasma membrane of the sinusoidal endothelial cell is flattened and pseudopods and vesiculations are hardly seen. The mitochondria are decreased in number and size. In DISSE's space, nothing is discovered.

2) Hepatic Parenchymal Cell (Plate 17)

Fat droplets of about 1 μ in size decrease in number and only one or two fat droplets are usually observed in the hepatic parenchymal cell. Lipid particles are also rarely discovered anywhere. Mitochondria locate around fat droplets, and under high magnification it is observed that mitochondria are in contact with the fat droplet showing an attitude as if it were covering the droplet. Therefore, mitochondria exhibit a curved form against the fat droplets (Plate 18). The contact surface of the fat droplets with mitochondria becomes smoothed and then obscured. On the other hand, the mitochondrial outer layer on the contact surface disappears completely and mitochondrial crests are arranged in such a manner as they are placed vertically to the contact surface. Furthermore, the electron density of the mitochondrial crests seems to be somewhat increased in

this stage.

(6) Six Hours after the Intravenous Administration of Thoracic Chyle

1) Sinusoidal Lumen and Sinusoidal Endothelial Cell (Plate 19)

Except for one or two erythrocytes, nothing is seen in the sinusoidal lumen. The sinusoidal endothelial cell shows a spindle-like form and its plasma membrane is smooth and devoid of pseudopods. The electron density of the cytoplasm is decreased, and mitochondria and endoplasmic reticulum are diminished in number. The nucleus is small and placed eccentrically in the peripheral zone of the cell, and the GOLGI complex is indistinct. DISSE's space is vacant as before.

2) Hepatic Parenchymal Cell (Plate 20 and 21)

In this stage, fat droplets are hardly found in the hepatic parenchymal cell and only one or two fat droplets are observed in surveying ten odd cells. Mitochondria increase strikingly in number and 50～60 in one hepatic parenchymal cell on an average are recognized being distributed evenly in the cell. Endoplasmic reticulum containing RNA-granules are distributed in the perinuclear and peripheral zone of the cytoplasm. Microbodies decrease in number and size in this stage.

IV. SUMMARY AND DISCUSSION

At the present time, it is generally acknowledged that, in the digestive tract, some of the fats which are orally taken, are in the non-hydrolyzed form (triglyceride), some in the partially hydrolyzed form (di- and mono-glyceride), and some in the form of isolated free fatty acids. They are reduced to corpuscles less than 0.5 μ in diameter by bile salts and can be absorbed into the thoracic duct through the intestinal mucous membrane. However, a part of the isolated free fatty acids which are formed as a result of hydrolysis is chemically changed into a water soluble compound and enter into the intestinal mucous membrane. Finally, this compound forms phospholipid which can be absorbed into the thoracic duct, too.

In fact, as shown on Table 1, besides glyceride which forms 76.4% of lipids in the thoracic chyle of cats, phospholipid is contained at the rate of 14.9% in the chyle. But, previously NAKAMURA in our laboratory, studying electron microscopically the intrahepatic metabolic process of fat after the intravenous administration of triglyceride emulsion made from sesame oil, arrived at the following conclusion: The droplets of triglyceride are first taken into the hepatic sinusoidal endothelial cell according to the same manner which was described by R. J. BENNET as the "concept of transportation by vesiculation of the membranes". And then, the droplets go through a primary change under the action of the mitochondria and are transformed into phospholipid. These droplets of phospholipid disappear from the sinusoidal endothelial cell, and next, in the form of α-lipoprotein, are transfered by the blood stream into DISSE's space always through the small pores of sinusoidal endothelium. Furthermore, droplets enter into the hepatic parenchymal cell and may go through a secondary metabolic change. However, it could not be said positively that the above-mentioned experimental results of NAKAMURA are the physiological process of chylomicra, i. e. droplets of glyceride which were administered orally and entered into the blood stream via the intestinal mucous membrane and the thoracic duct.

Therefore, the author attempted to clarify this problem by the preceeding methods and materials. Namely, in this experiment, instead of the method of NAKAMURA in which artificially made triglyceride emulsion was injected intravenously, the author observed electron microscopically the intrahepatic metabolic process of chylomicra of experimental animals to which triglyceride emulsion was first administered orally and thoracic chyle, being collected from the thoracic duct of the same individual, was next given intravenously. Althogh, many fat droplets of 0.5～1.0 μ in diameter have already appeared in the hepatic parenchymal cell 10 minutes after the intravenous administration of thoracic chyle, these fat droplets are considered to be phospholipid which was contained in the thoracic chyle at the rate of 14.9% and transfered by the blood stream always into DISSE's space through the small pores of sinusoidal endothelium and entered into the hepatic parenchymal cells. This process is thus completely in accordance with the fact which had been verified histochemically by SHIROTANI (1957) in our laboratory. However, as previously clarified by NAKAMURA, fat droplets are not observed in the hepatic parenchymal cell immediately after the intravenous injection of glyceride emulsion and they appear for the first time in the cell after 2 or 3 hours following injection, and furthermore, in the thoracic chyle used in this experiment, not only glyceride, but also phospholipid is contained simultaneously. On the contrary, only glyceride was contained in the emulsion used in NAKAMURA's experiment. It is reasonable, therefore, to consider that fat droplets which appeared immediately after the intravenous injection of thoracic chyle are the droplets of phospholipid. Namely, phospholipid is directly taken into the hepatic parenchymal cell immediately after injection, but on the contrary, glyceride is not taken directly. These facts support the experimental results of FISHLER and his co-workers that when phospholipid labeled with P^{32} and C^{14} was injected intravenously the majority of it was transfered immediately into the hepatic parenchymal cell in the unchanged form. But, phospholipids which appeared immediately in the hepatic parenchymal cell have vanished 30 minutes after injection and cannot be oberved electron microscopically anywhere. Because of this, it is considered that this phospholipid is treated and utilized according to the metabolic process which will be mentioned later.

On the other hand, glyceride, which occupy 76.4% of lipids contained in intravenously administered thoracic chyle, are transfered by blood circulation to the sinusoidal lumen in the form of 0-lipoprotein i. e. chylomicra of 0.15～0.5 μ in size as confirmed by FUJINO and are taken into the sinusoidal endothelial cell. Concerning these process, NAKAMURA reported electron microscopically the observation that these chylomicra were not caught by pseudopods of the sinusoidal endothelial cell but being surrounded, several as a group, by the encircling membrane formed by vesiculation of the plasma membrane, they were taken into the cell.

In 1956, BENNET advocated the concept of transportation by vesiculation of the membranes. According to this concept, this vesiculation may be an important mechanism carrying particles and molecules as well as ions, into and out of the cells and has such important physiologic activities of the cell as permeability, circulation of substances, synthesis, and storage within membranes, secretion and so forth. Chylomicra taken into the sinusoidal endothelial cell by such a mechanism begin to coalesce each other into

larger fat droplets 30 minutes after injection and in turn are exposed into the protoplasm after losing their encircling membranes. Then mitochondria come together around these larger fat droplets. One hour after the intravenous injection of thoracic chyle these larger fat droplets begin to contact functionally with mitochondria, and the mitochondrial outer layer on the contact surface becomes obscure. During this process, mitochondrial crests are placed at right angles to the contact surface with fat droplets. The above-mentioned process is the same as that reported by NAKAMURA previously, and it is considered that glyceride, i. e. chylomicra transported into the sinusoidal endothelial cell by this process, is changed to phospholipid under the action of the mitochondria. On the other hand, E. P. KENNEDY and his associate have instituted an inquiry into the formation process of phospholipid, using the fraction of mitochondria separated by ultracentrifugation from the hepatic tissue homogenated, and succeeded in actual demonstration of the formation of phospholipid from glyceride under the action of mitochondria. Consequently, the reason why we have arrived at the conclusion that phospholipid is biosynthesized from glyceride in the sinusoidal endothelial cell depends upon the fact that the above-mentioned larger fat droplets are changed into lipid particles of 300 Å in size under the action of mitochondria, in other words, under the action of enzymes. Thus, these lipid particles are corresponding with the size of the phospholipid participating in the formation of α-lipoprotein in the blood. By the time the larger fat droplets existing in the sinusoidal endothelial cell begin to contact functionally with mitochondria, many lipid particles of 300 Å in size are observed in the protoplasm on the side of DISSE's space, and next, these lipid particles, several as a group, are encircled by a kind of membrane of unknown origin and transported to DISSE's space. Accordingly, numerous lipid particles of 300 Å in size are observed in DISSE's space, and a part of these particles is transfered into blood stream from DISSE's space through the pores of the sinusoidal endothelium and then recognized as the increase of α-lipoprotein in the blood, as verified biochemically by FUJINO.

In the meantime, the other part of the lipid particles in DISSE's space are surrounded, several in a group, by encircling membranes formed by vesiculation of the surface of microvilli and they enter directly into the hepatic parenchymal cell, in which they coalesce within the encircling membrane to become larger particles After the encircling membranes have disappeared, these larger lipid particles further coalesce and finally form fat droplets of about 1 μ in diameter. Moreover, it seems that these fat droplets are exactly the same as the fat droplets which have appeared in the hepatic parenchymal cell 10 minutes after the intravenous injection of thoracic chyle, in any finding such as form, size, electron density, and so on. From this point of view, it is also reasonable to consider, that the glyceride is converted into phospholipid under the action of mitochondria in the sinusoidal endothelial cell.

In short, on the occasion of intravenous administration of thoracic chyle, fat droplets appear in the hepatic parenchymal cell biphasically as previously demonstrated histochemically by SHIROTANI, while in the case of intravenous administration of glyceride emulsion, fat droplets appear only monophasically. Except for the behavior of the fat droplets which appeared in the hepatic parenchymal cell immediately after the intravenous injection of thoracic chyle, the other intrahepatic metabolic process of fat in the case of thoracic

chyle administration is definitely the same as in the case of glyceride emulsion. From these standpoints, glyceride contained in the thoracic chyle is treated once in the sinusoidal endothelial cell primarily and then transported into the hepatic parenchymal cell through DISSE's space. Even if it is the glyceride absorbed physiologically from the intestinal mucosa, it cannot enter directly into the hepatic parenchymal cell. In comparison with the experimental results of NAKAMURA, fat droplets which appeared in the hepatic parenchymal cell are considered certainly to be possessing the characteristics of phospholipid. It should be believed, therefore, that the site in which phospholipid is biosynthesized from glyceride, is in the sinusoidal endothelial cell. The behavior of mitochondria in the presence of fat droplets in the hepatic parenchymal cell 2 or 3 hours after injection, is entirely the same as in the case of 10 minutes after injection. Mitochondria come together around fat droplets and in functional contact with them, and at the same time, the mitochondrial outer layer contacting with the droplets disappears. Moreover, mitochondrial crests are placed at right angles to the contact surface. Finally, fat droplets, when almost completely treated and utilized, are hardly observed in the hepatic parenchymal cell. It is believed that fat droplets may be oxidized and utilized under the action of mitochondria in the hepatic parenchymal cell. On the other hand, the author recognized a very interesting fact which will be described as follows: Microbodies in the hepatic parenchymal cell begin to increase in number and size and a double membranous structure is clearly recognized following intravenous administration of fatty chyle. Furthermore, in the peripheral part of the microbodies, a fine concentric stratified structure was observed, and in the center several fine globular cores of 1,300～500 Å in size with high electron density were also observed. From the time when mitochondria of the hepatic parenchymal cell begin to decrease in number after showing an important role in the metabolic process of fat droplets, microbodies begin to increase in number and in size, vice versa, and become as large as or far larger than mitochondria. Sometimes, a mitochondrial crest-like structure is observed in the microbodies. Therefore, these microbodies are indistinguishable from mitochondria and are considered to be in an intermediate stage between microbodies and mitochondria. This finding suggests the process in which microbodies are transformed into mitochondria for the purpose of replacing decreased mitochondria.

Microbodies were first described by RHODIN (1954) in the proximal tubule of the kidney and subsequently studied in the liver, in a different experimental condition by ROUILLER and BERNHARD (1956). They found that the microbodies increase considerably in number in the regenerating liver after hepatectomy or poisoning with drugs that destroy liver cells (carbon tetrachloride), and feeding after fasting, thus advocating the hypothesis that microbodies are the precursor of mitochondria. The author believe that these experimental results support their hypothesis. But, on the other hand, GANSLER and ROUILLER also reported that the formation of mitochondria is progressed by the fragmentation of long mitochondria into smaller oval or spherical ones. In the present time, however, further investigation should be made concerning the intracytoplasmic function of microbodies.

V. CONCLUSIONS

After oral administration of triglyceride emulsion to adult cats at a rate of 4 g of

fat per kg of body weight in the post-absorptive state, thoracic chyle was collected from the thoracic duct. The intrahepatic metabolic process of fat was studied electron microscopically after the chyle was administered intravenously to the same cat from which it was collocted. The following conclusions were then obtained :

(1) Phospholipid contained in the thoracic chyle in the ratio of about 15% is transfered directly into the hepatic parenchymal cell through DISSE's space by the blood stream and went through metabolic changes. However, the sinusoidal endothelial cell is not involved in this process at all.

(2) Glyceride contained in the thoracic chyle is first transported into the sinusoidal lumen in the form of 0-lipoprotein by the blood stream and then taken into the sinusoidal endothelial cell, in which it goes through a primary metabolic change by the action of the mitochondria and is converted into phospholipid. Finally this phospholipid is transfered into the hepatic parenchymal cell through DISSE's space. That is to say, chylomicra, i. e. droplets of glyceride, are taken into the sinusoidal endothelial cell being surrounded, several in a group, by the encircling membrane which was formed from the vesiculation of the plasma membrane. Then, chylomicra coalesce into larger fat droplets within the encircling membrane and, furthermore, these fat droplets are directly exposed in the protoplasm after the encircling membrane has disappeared. Mitochondria are in functional contact with these fat droplets in this stage and the mitochondrial outer layer facing on the contact surface disappears. At the same time, mitochondrial crests are placed at right angles to the contact surface. Thus, these fat droplets become irregular in shape, indistinctly bordered and disappear at last. At the same time, lipid particles of 300 Å in size begin to appear in the protoplasm facing on DISSE's space. These lipid particles are surrounded by a membrane of unknown origin and then are discharged into DISSE's space.

(3) Accordingly, numerous lipid particles of 300 Å in size are observed in DISSE's space and a part of these particles goes into the blood circulation from DISSE's space through the small pores of the sinusoidal endothelium. It is considered that these particles in the blood stream are recognized as the increase of α-lipoprotein in the blood as previously reported by FUJINO. The other part of the lipid particles which appeared in DISSE's space is taken into the hepatic parenchymal cell being surrounded, several in a group, by the encircling membrane originated from vesiculation of the microvilli and they coalesce into larger particles within the encircling membrane. Then, these larger lipid particles are exposed in the protoplasm after the encircling membrane has disappeared, and they further finally coalesce progressively into larger fat droplets of about 1 μ in diameter.

(4) The amount of fat transfered into the hepatic parenchymal cell reaches its maximum 2～3 hours after the intravenous administration of thoracic chyle and then fat droplets are oxidized and utilized under the action of the mitochondria and go out of sight.

(5) As clarified histochemically by SHIROTANI in our laboratory, it is confirmed electron microscopically that the behavior of fat appeared in the hepatic parenchymal cell is biphasic on the occasion of the intravenous injection of thoracic chyle.

(6) In short, giving consideration to the experimental results of NAKAMURA and FUJINO, it is considered that glyceride cannot enter directly into the hepatic parenchymal

cell and primary enzymologic activity in the sinusoidal endothelial cell under the action of mitochondria is quite indispensable for glycerides, to enter into the hepatic parenchymal cell. Therefore, to clarify all the phenomena observed in this experiment, it should be considered that glyceride is once changed into phospholipid in the sinusoidal endothelial cell under the action of the mitochondria.

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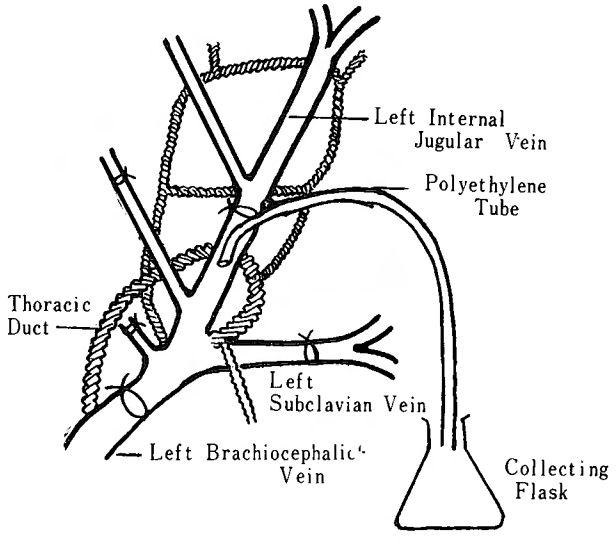
EXPLANATION OF PLATES

BC : Capillary bile duct
C : Chylomicron
CF : Collagen fiber
CM : Cell membrane
DS : Disse's space
EM : Encircling membrane
ER : Endoplasmic reticulum
F : Fat droplet
FR : Ferritin granule
LC : Liver parenchymal cell
LP : Lipid particles
LV : Lymphatic vessel
M : Mitochondria
MB : Microbody
MN : Mitochondrial membrane
MV : Microvilli
NC : Nucleus
NCL : Nucleolus
NM : Nuclear membrane
P : Pore
PP : Pseudopod
RBC : Red blood cell
SE : Sinusoidal endothelium
SEC : Sinusoidal endothelial cell
SL : Sinusoidal lumen
V : Vesicle

Fig. 1 Method of collecting chyle



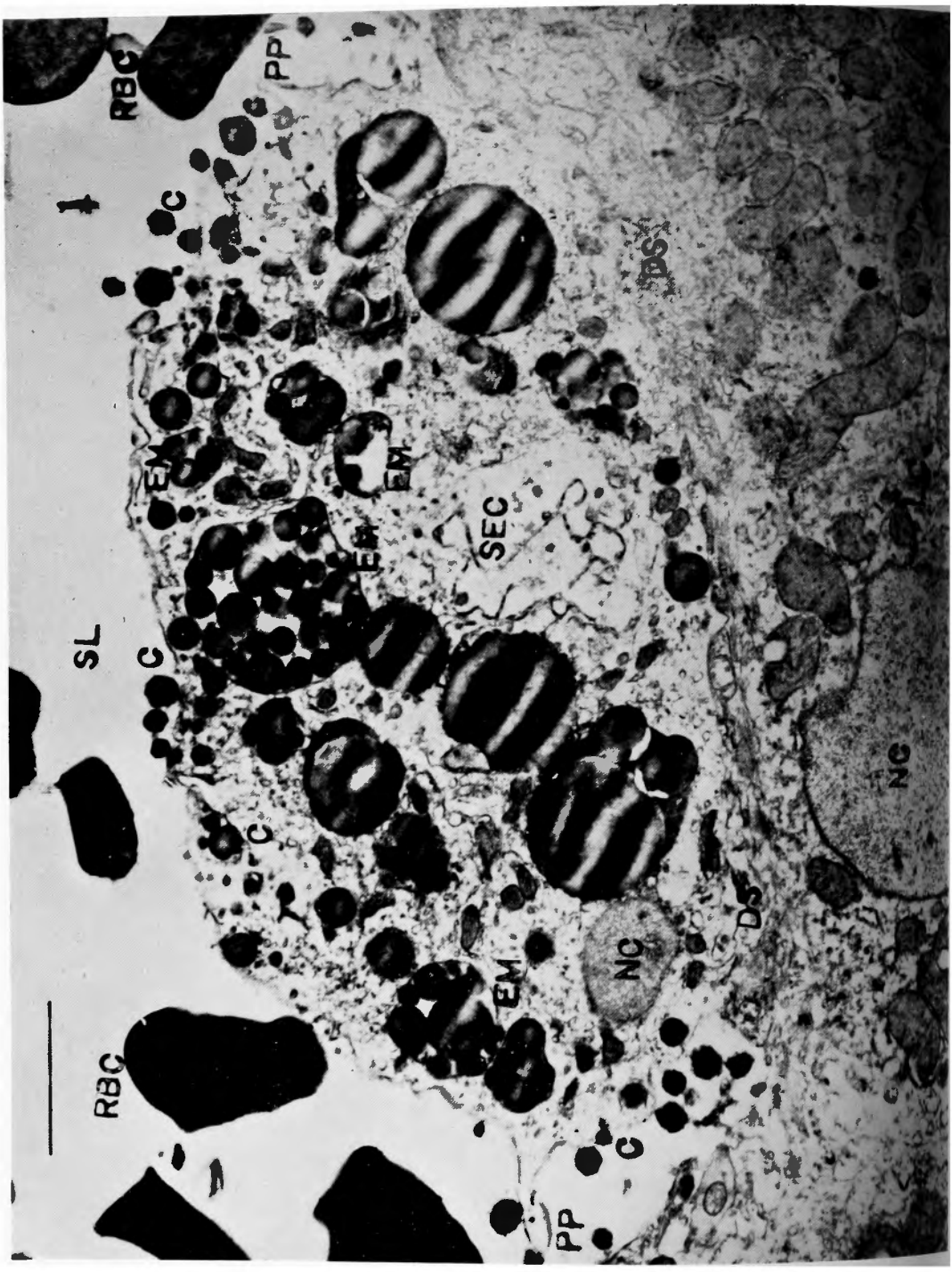


Plate 1. A sinusoidal endothelial cell (10 minutes after injection)

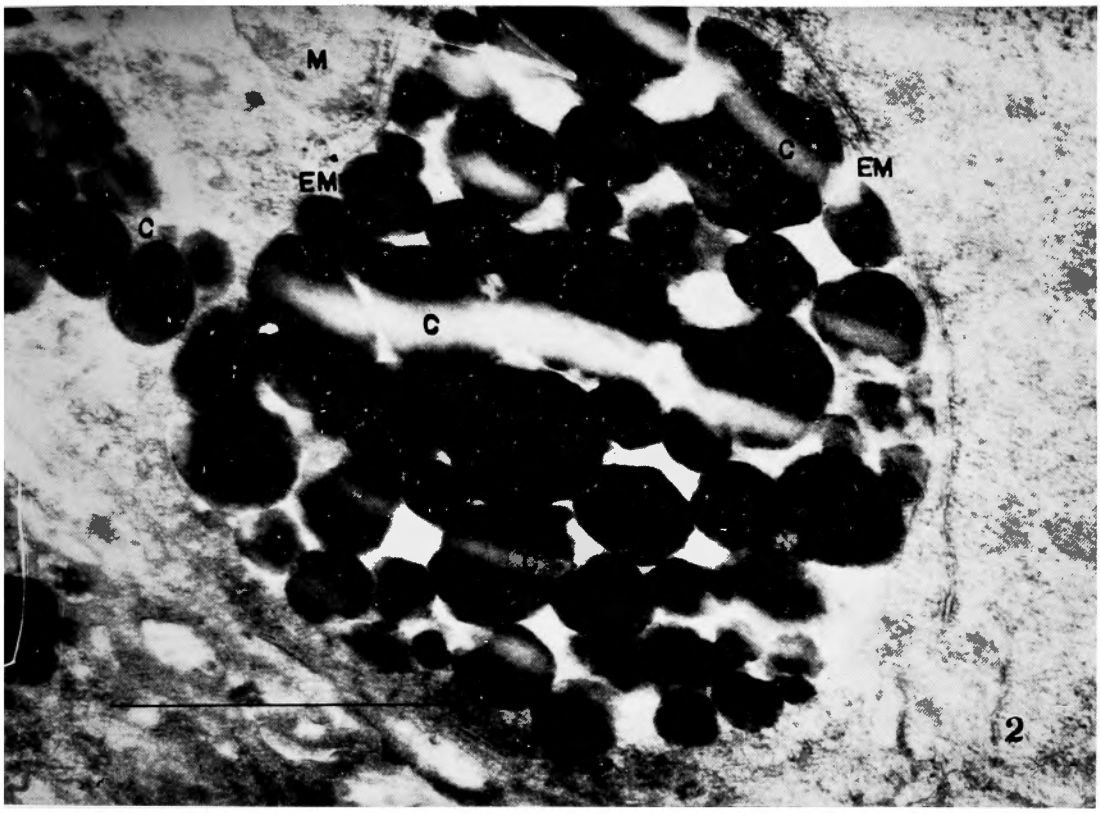


Plate 2. Chylomicra surrounded by the encircling membrane under high magnification.

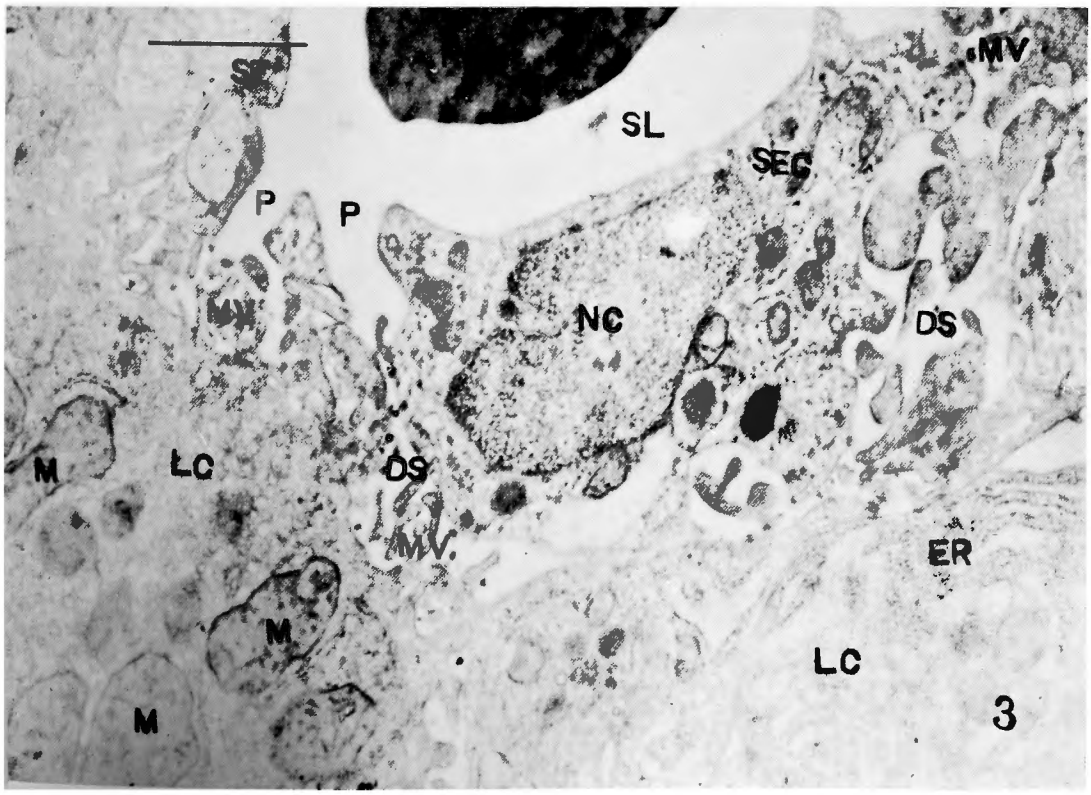


Plate 3. Structure of the hepatic sinusoid.

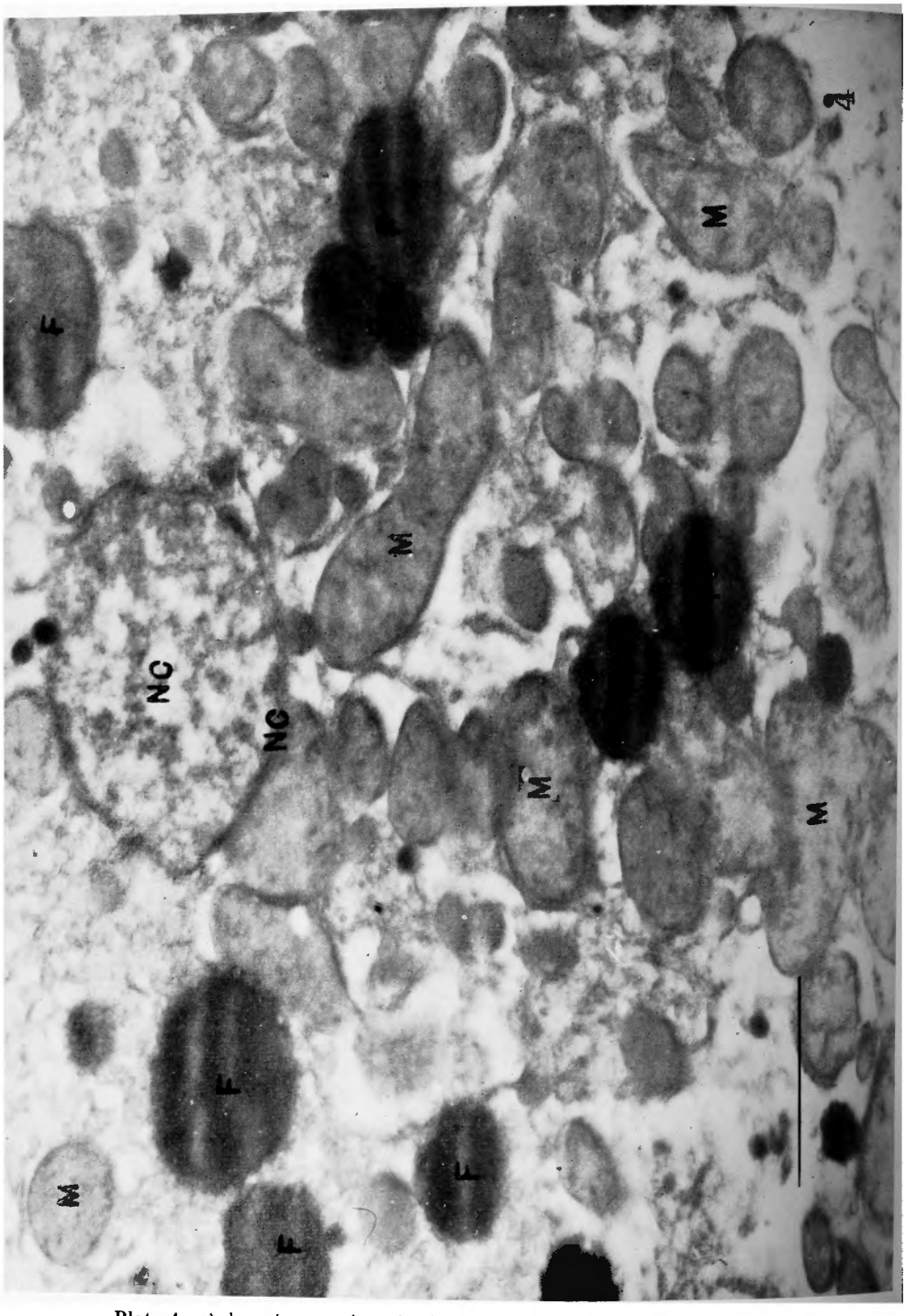


Plate 4. A hepatic parenchymal cell (10 minutes after injection)

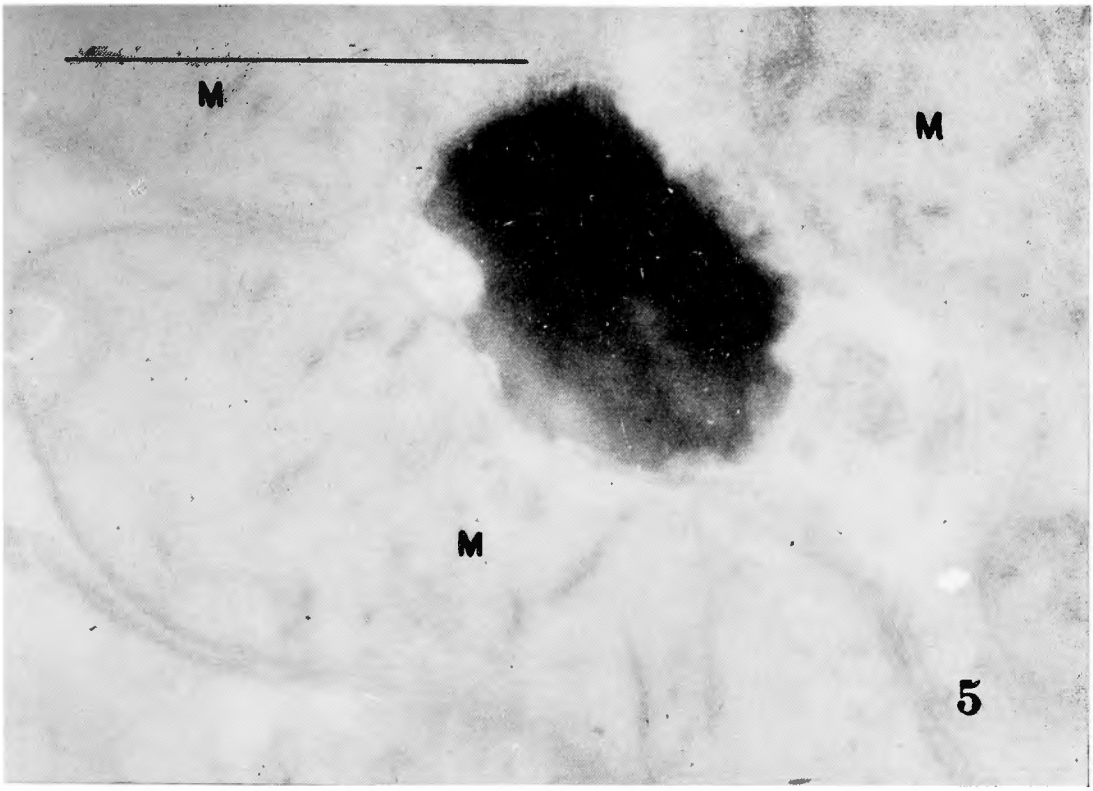


Plate 5. A mitochondrium of the hepatic parenchymal cell and a fat droplet (10 minutes after injection)

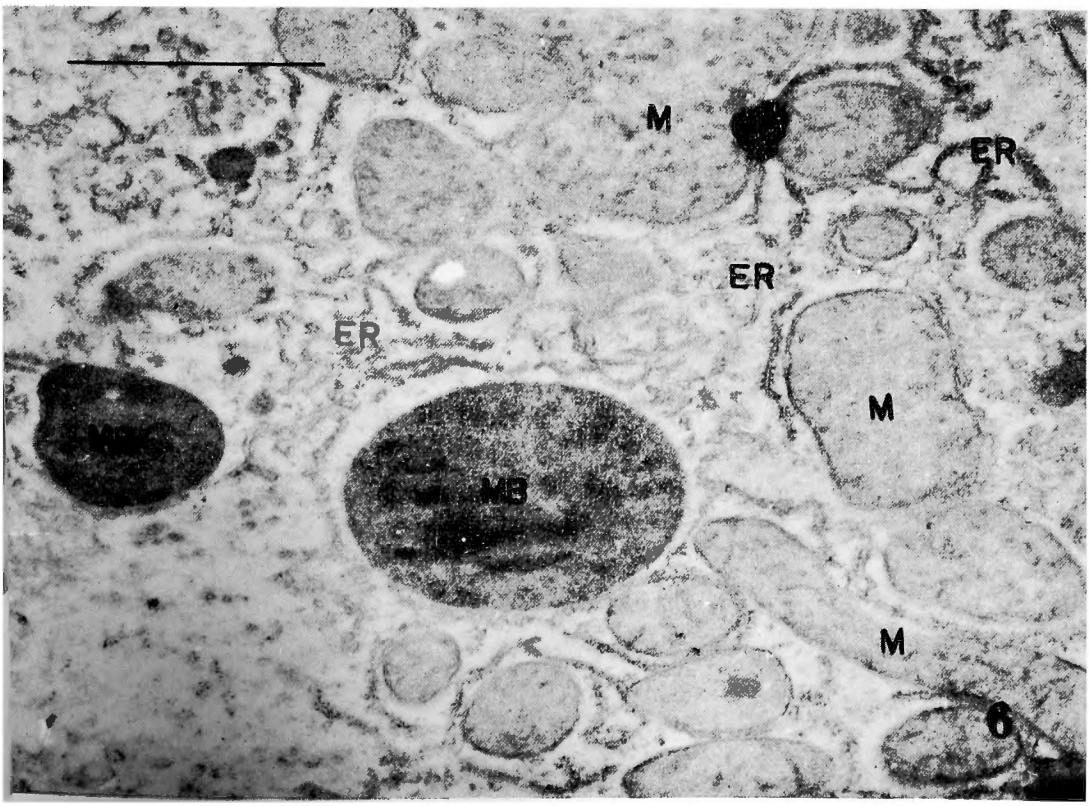


Plate 6. Microbodies in the hepatic parenchymal cell (10 minutes after injection)

Plate 7. A sinusoidal endothelial cell (30 minutes after injection)

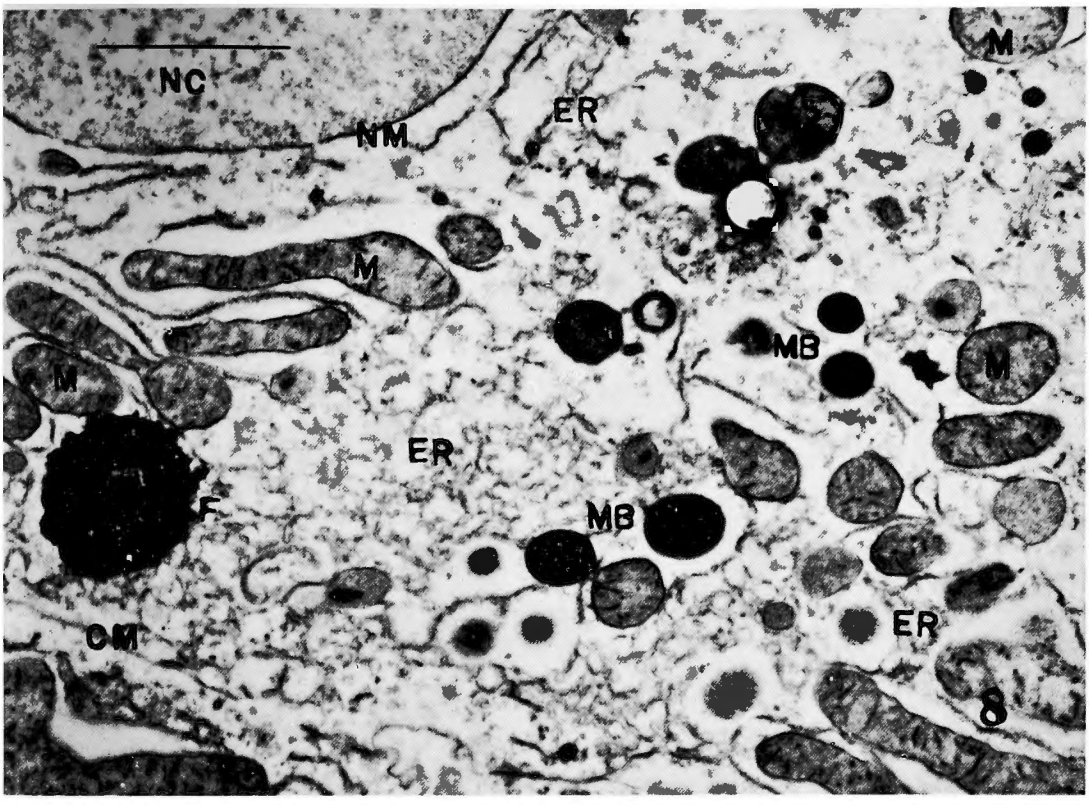


Plate 8. Hepatic parenchymal cell (30 minutes after injection)

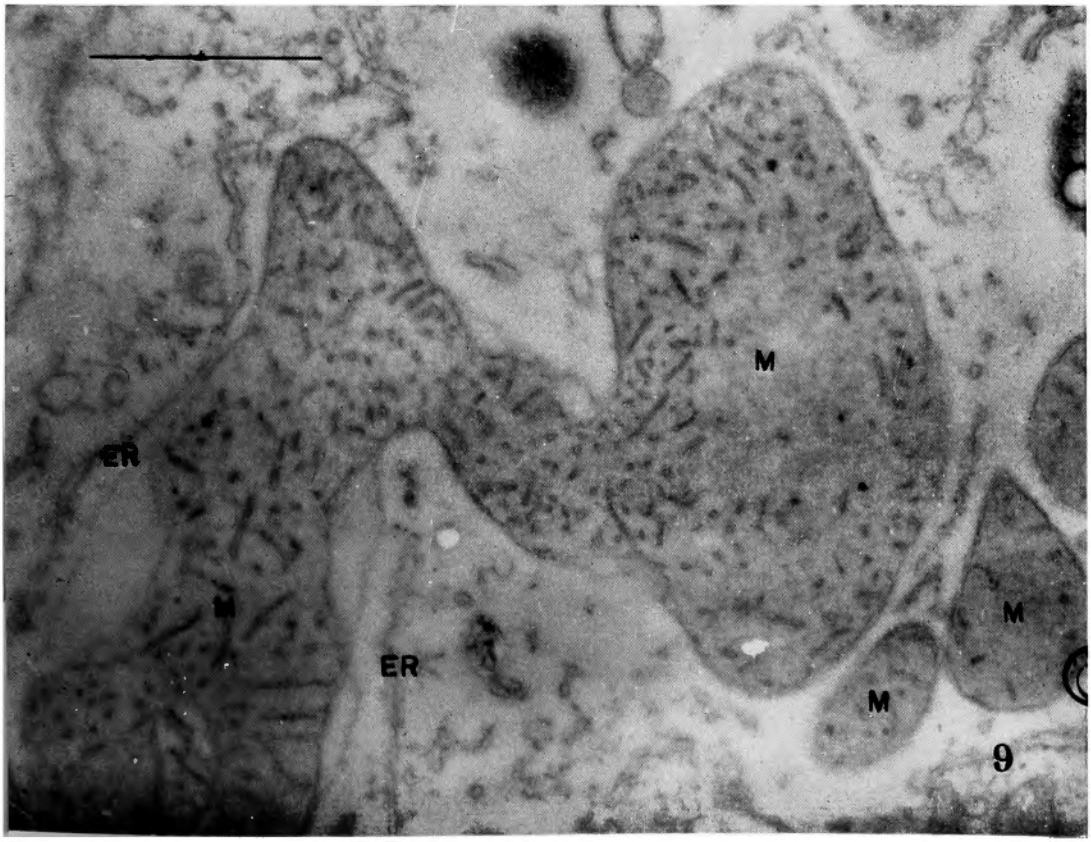


Plate 9. A large mitochondrium appeared in the hepatic parenchymal cell (30 minutes after injection)

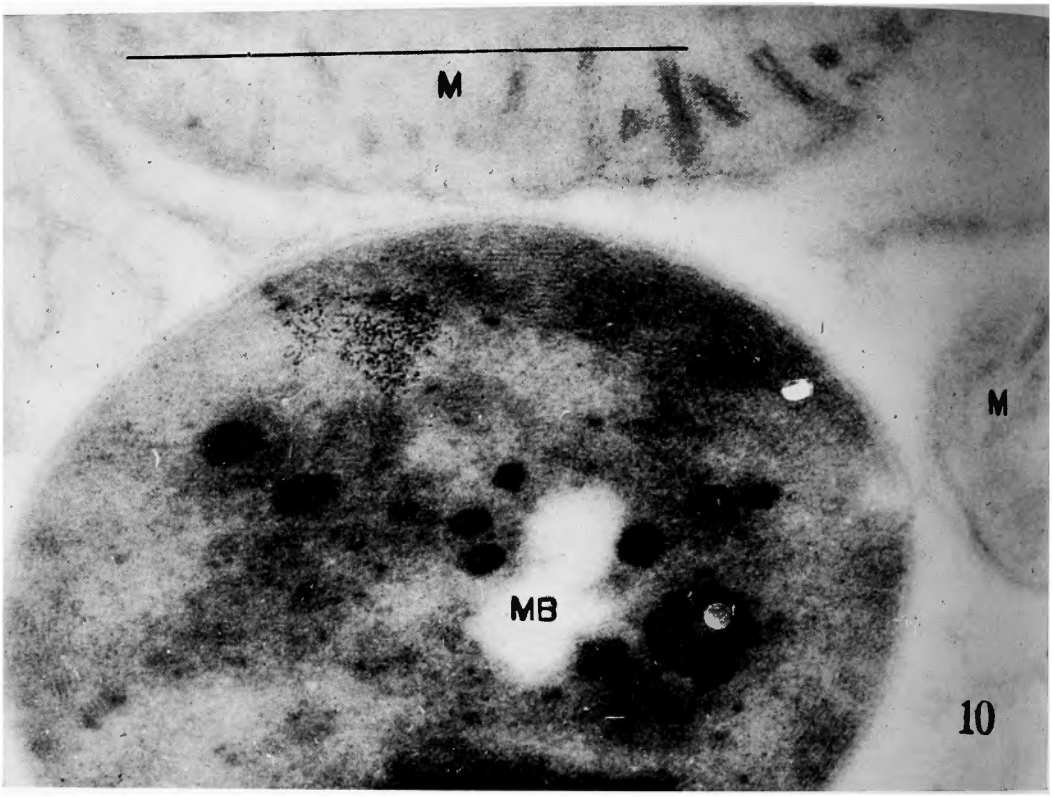


Plate 10. A microbody under high magnification (30 minutes after injection)

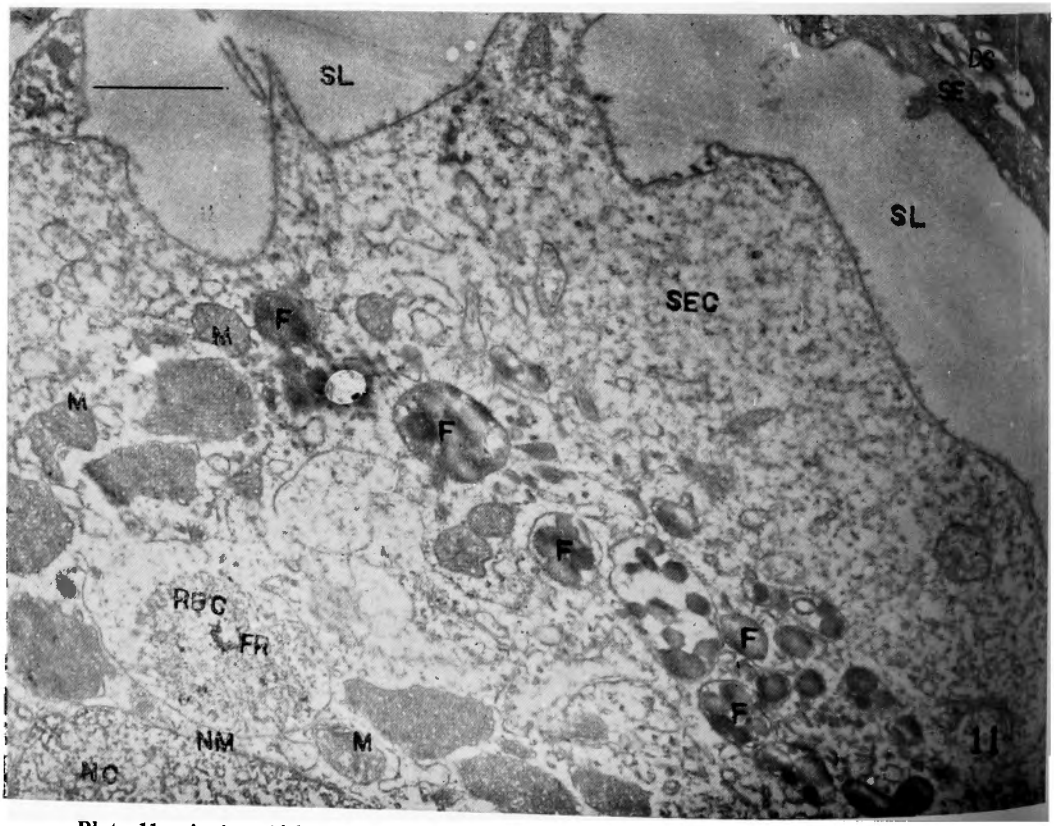


Plate 11. A sinusoidal endothelial cell (one hour after injection)

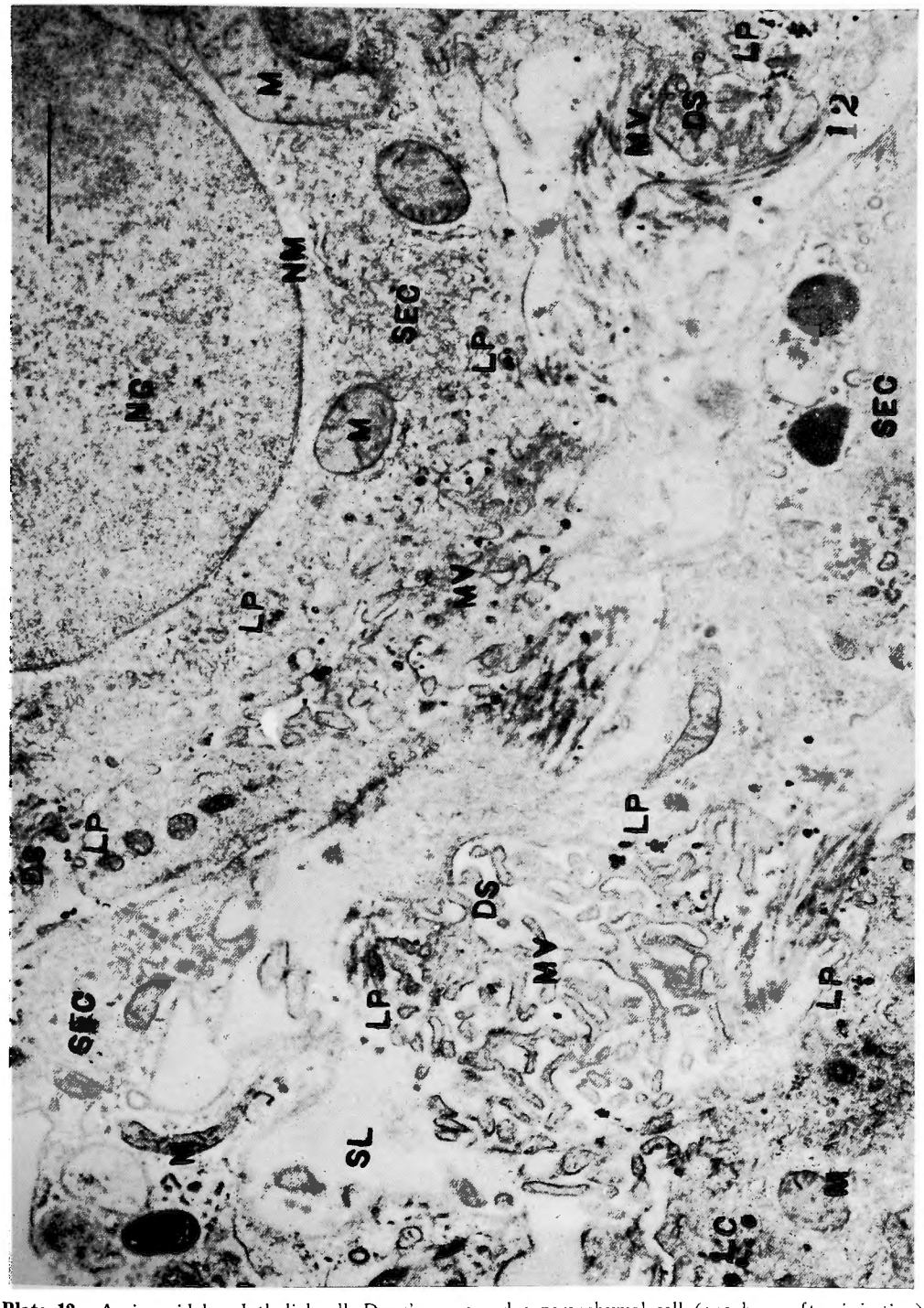


Plate 12. A sinusoidal endothelial cell, Disse's space and a parenchymal cell (one hour after injection)

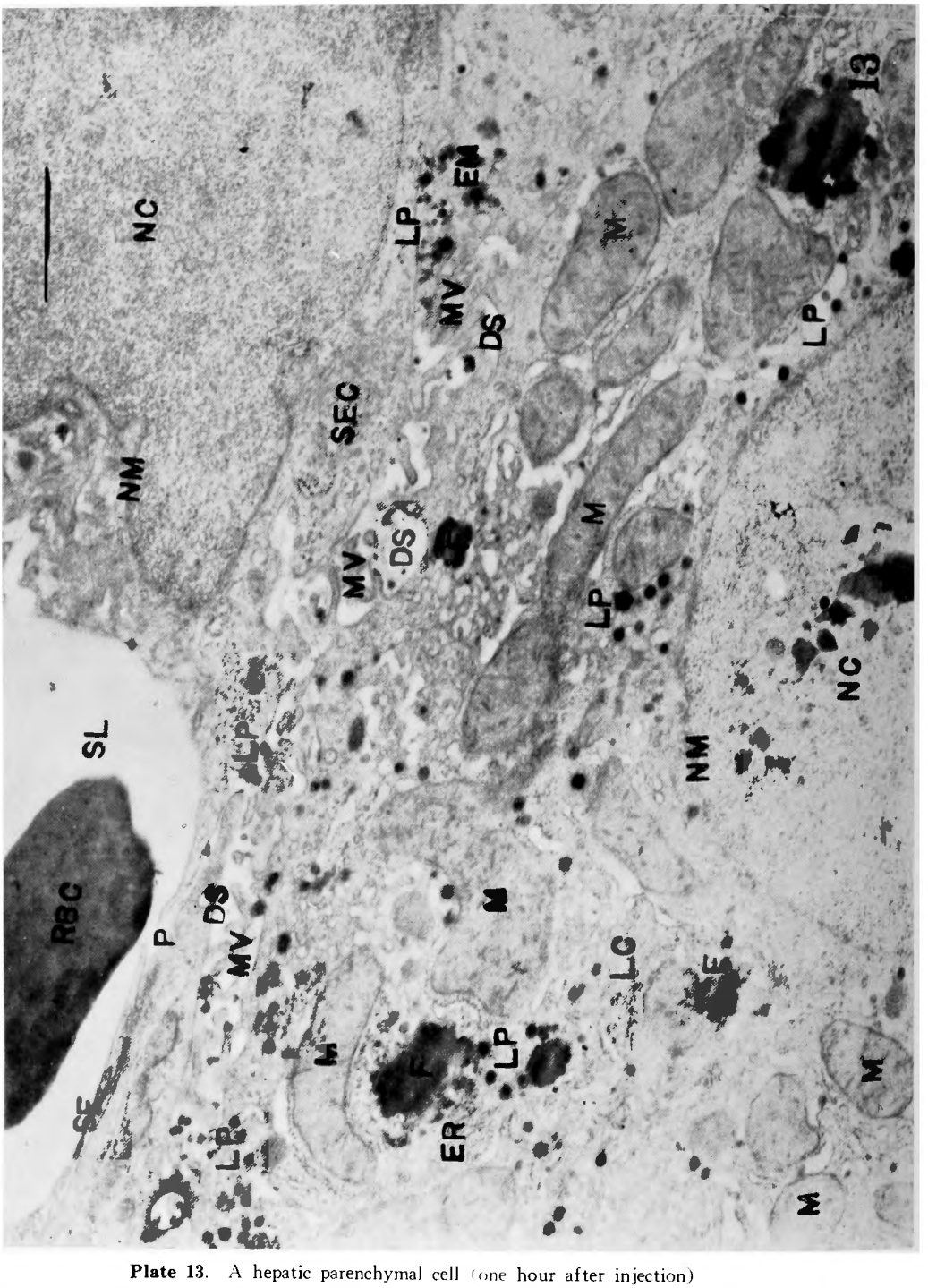


Plate 13. A hepatic parenchymal cell (one hour after injection)

Plate 14. A sinusoidal endothelial cell (2 hours after injection)

Plate 15. A hepatic parenchymal cell (2 hours after injection)

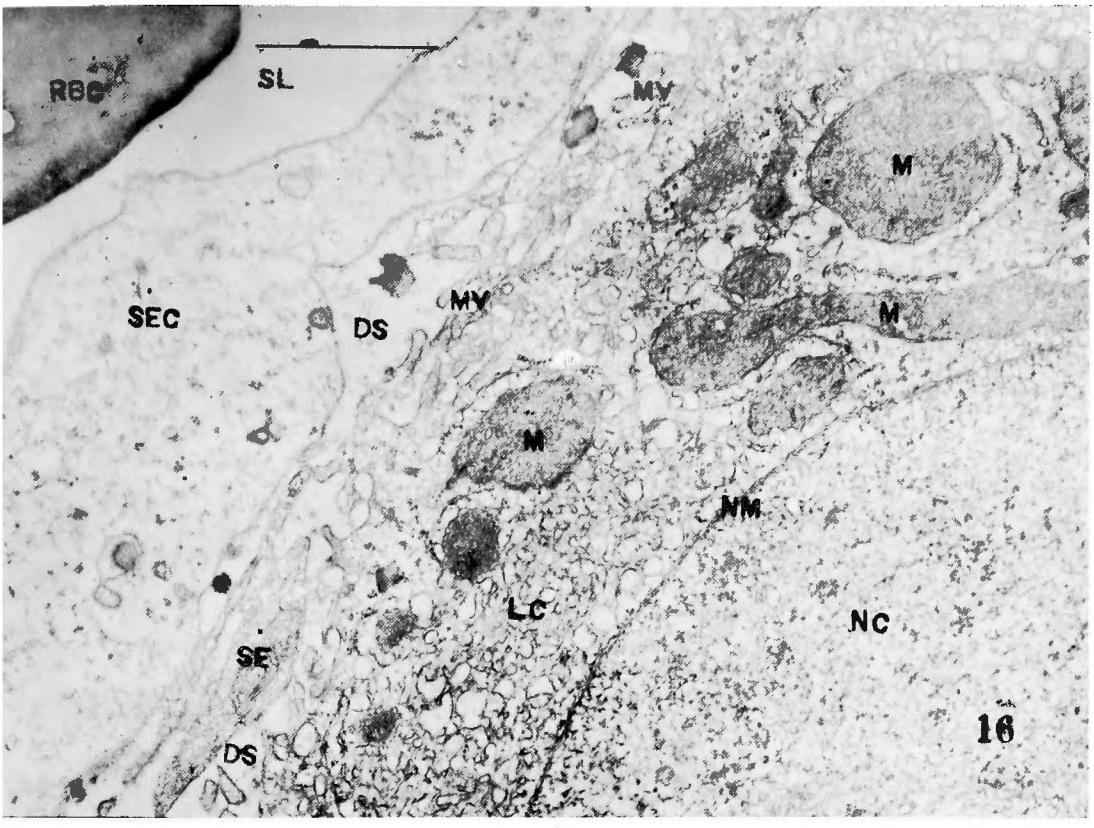


Plate 16. A sinusoidal endothelial cell (3 hours after injection)

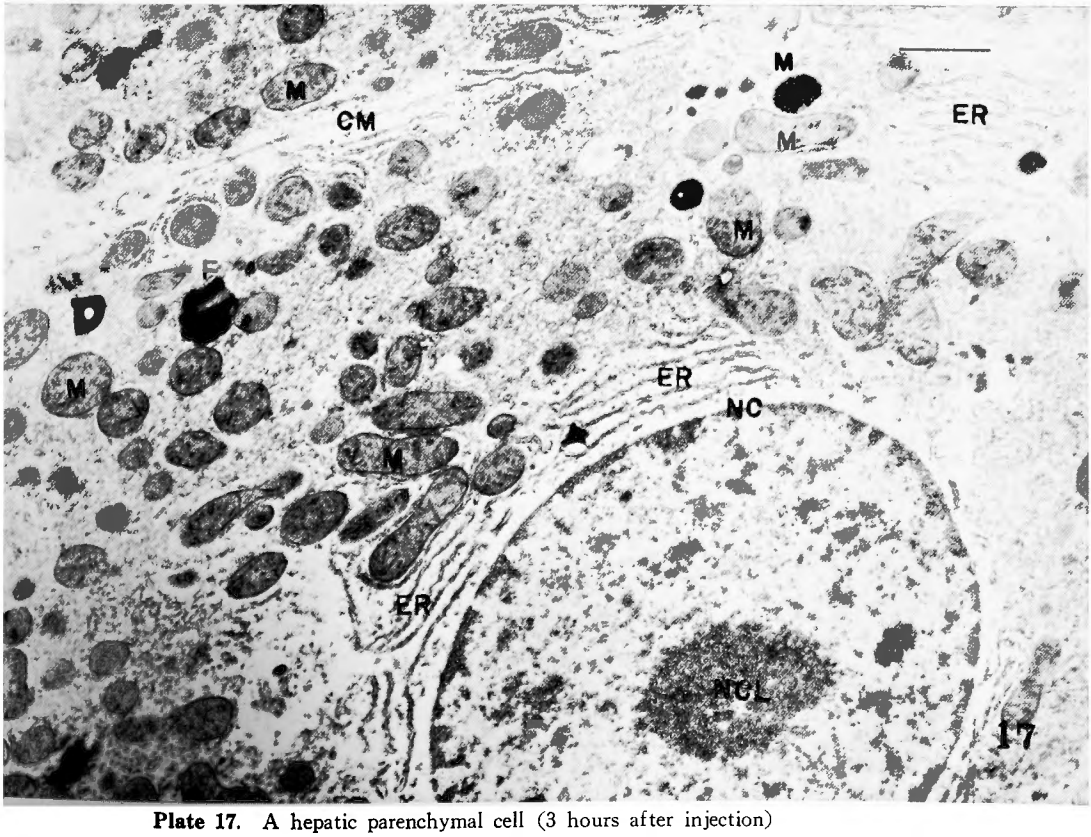


Plate 17. A hepatic parenchymal cell (3 hours after injection)

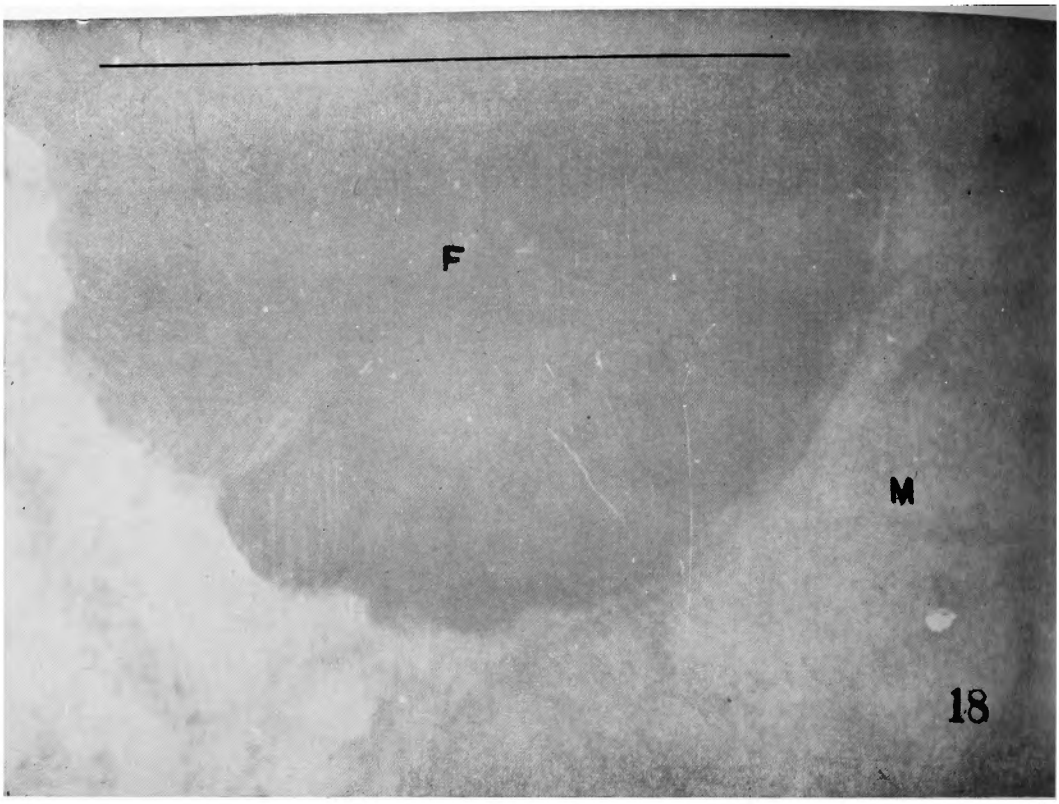


Plate 18. A fat droplet contacting with a mitochondrium in the parenchymal cell (3 hours after injection)

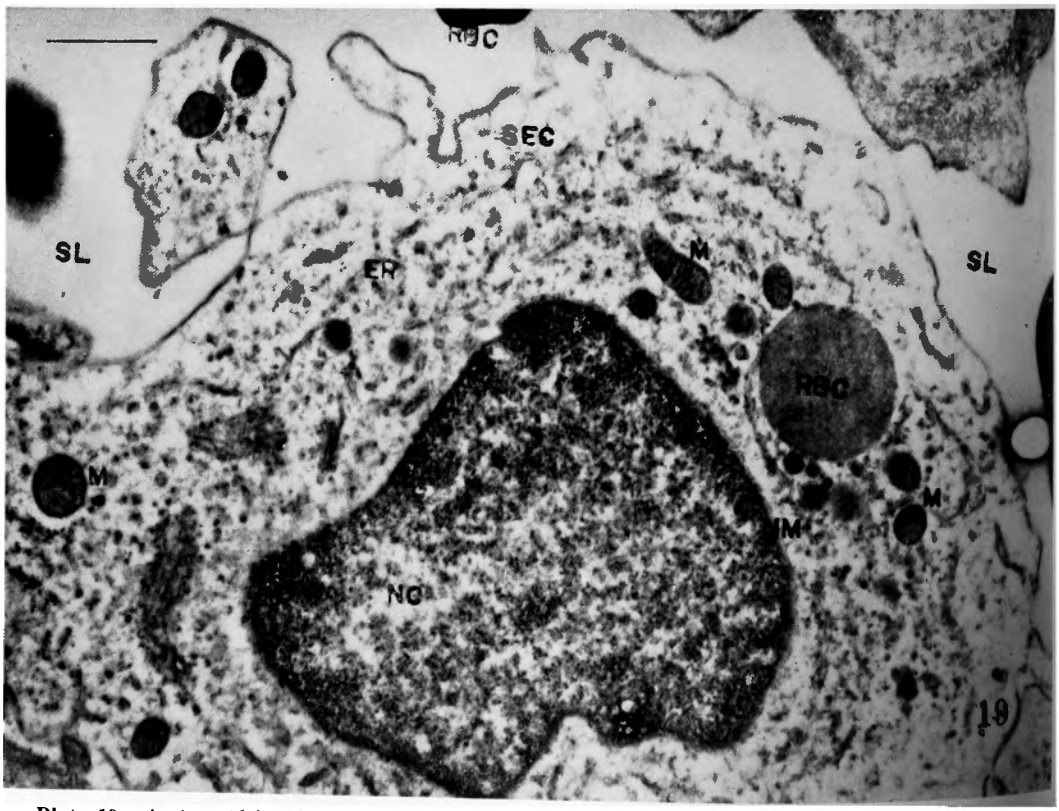
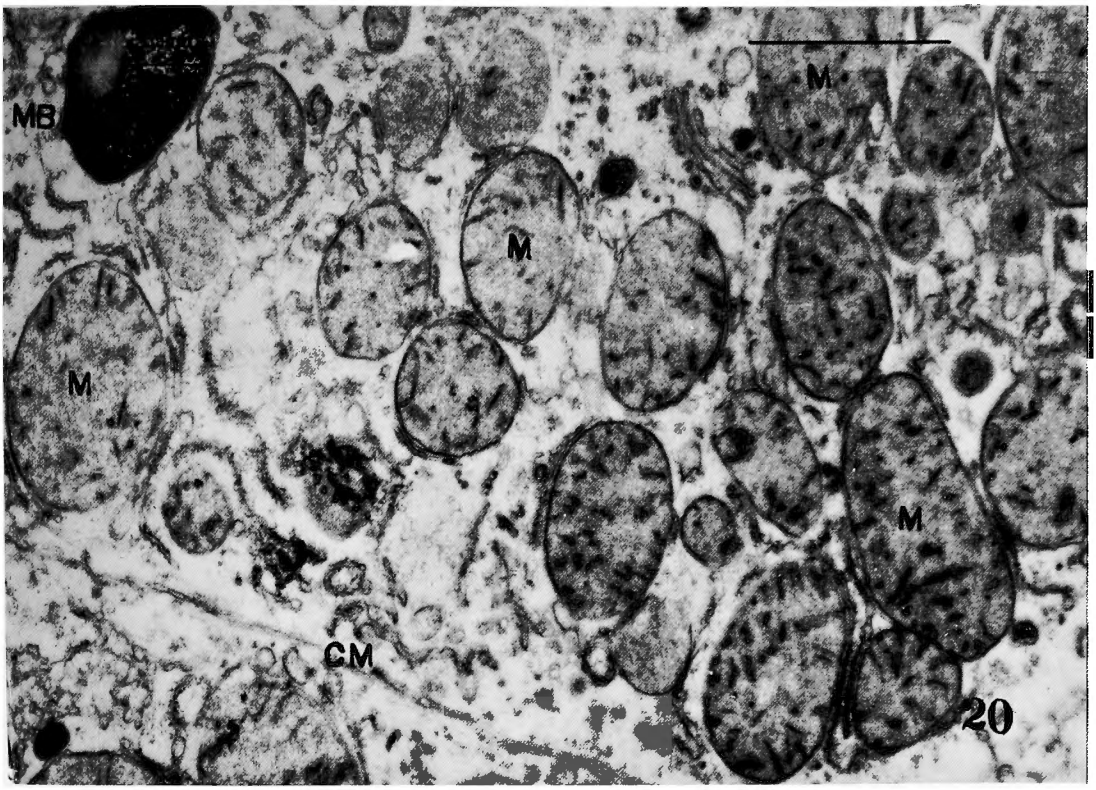


Plate 19. A sinusoidal endothelial cell (6 hours after injection)



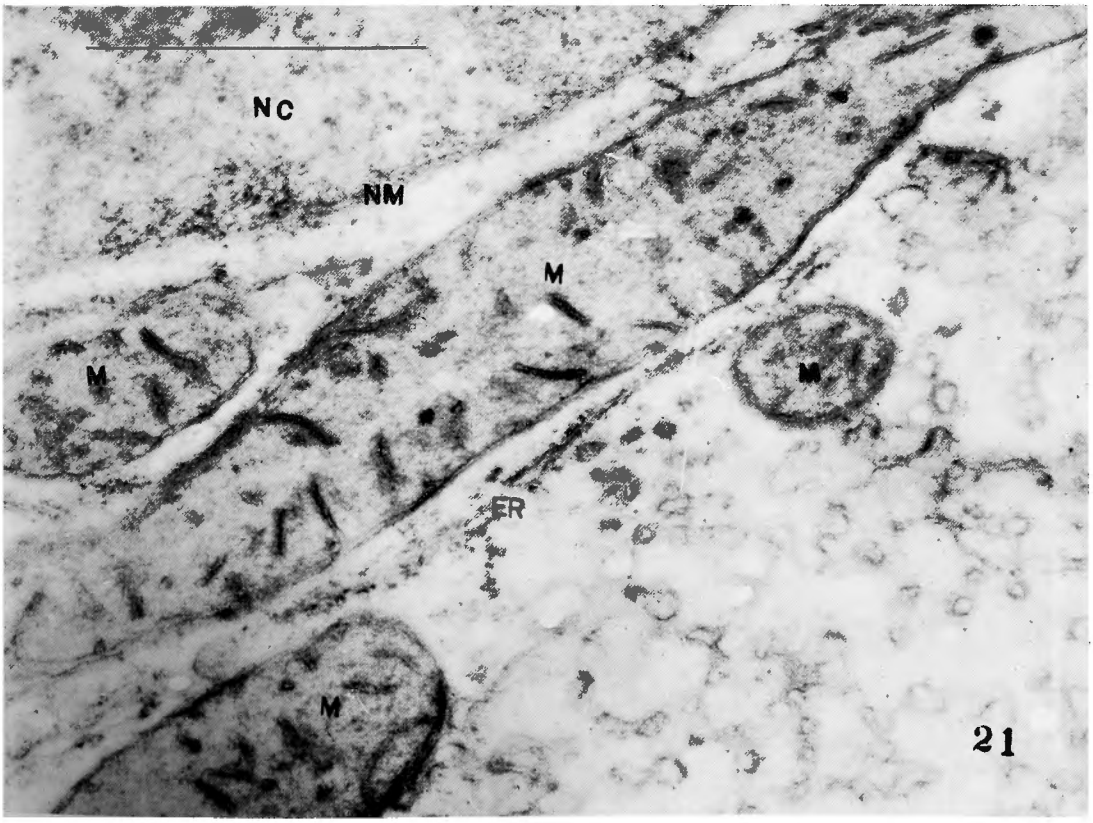
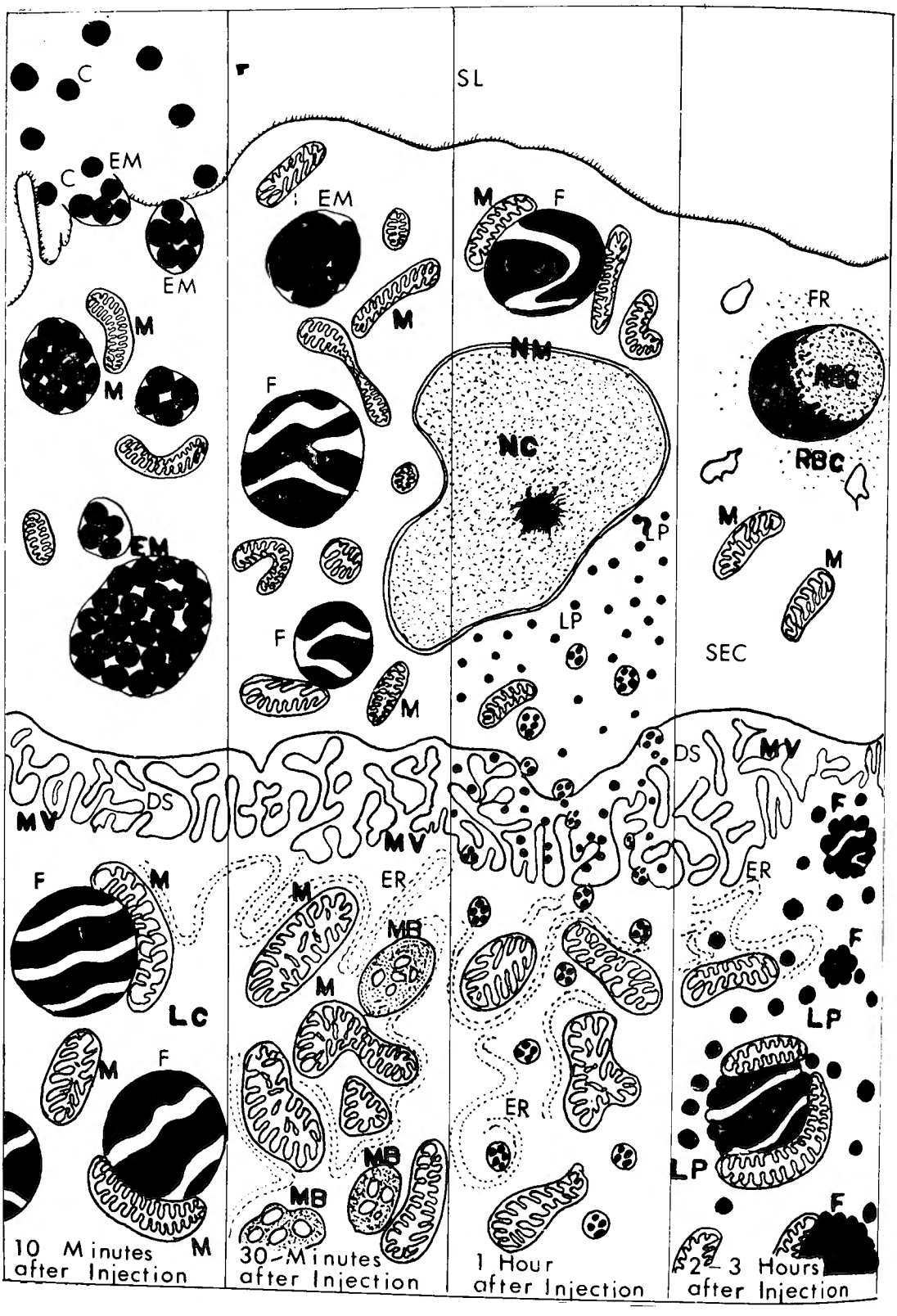


Plate 20 and 21. A hepatic parenchymal cell (6 hours after injection)

Plate 22. Schematic drawing showing the intrahepatic metabolic process of the thoracic chyle. The lapse of time after the administration is indicated in the horizontal dimension.



和文抄録

肝臓に於ける脂質処理能についての電子顕微鏡学的研究

京都大学医学部外科学教室第2講座 (指導：青柳安誠教授)

清 水 俊 丸

Post-absorptive Stateにした成熟猫に対し，体重毎kg当り4gの脂質を予め経口的に投与した後，当該個体から採取し得た胸管乳糜を更めて同一個体に静注し，その肝臓における脂質の処理過程を電子顕微鏡学的に追究し，次の結論を得た．

(1) 胸管乳糜中に比較的多量に含まれる約15%のPhospholipidは静脈内注入後10分で，すでに流血を介してDissé氏腔に入り，次いで肝実質細胞内に移行し処理されるが，この過程には静脈洞内被細胞は全く関与していない．

(2) 胸管乳糜中のGlycerideは，まずO-lipoproteinとして流血を介して静脈洞に移行し，そこに存在する内被細胞に摂取され，それら細胞内でMitochondriaの作用下に一次的処理を受けた後，Dissé氏腔を経て肝実質細胞内に移行する．即ちChylomicra—Glycerideの小滴は数個づつ内被細胞のPlasma membraneのVesiculationによつて生じたEncircling membraneに囲繞され摂取されて，それらはEncircling membrane内で融合して大きな脂質滴となる．そしてこの頃になるとEncircling membraneは消失して，大きな脂質滴は原形質内に直接露呈して存在するようになる．而もMitochondriaはこの脂質滴と機能的に接触するが，その際，接触面のMitochondriaの外膜は消失し，そのCrestsは接触面に対して直角になるような配列型式をとる．斯くして脂質滴は漸次不規則，不鮮明となり消失するが，これと同時に内被細胞のDissé氏腔に面した側の原形質内に直径300Å前後の脂質の微粒子が再び出現し，その数個づつが1単位となつて，その由来の不明な一種の膜によつて匿繞され，次いで，これはその脂質の微粒子をDissé氏腔に向けて放出する．

(3) それでこの頃になると，Dissé氏腔内には，直径300Å大の脂質の微粒子が沢山出現するが，その一部はDissé氏腔から内被の小孔を通つて流血中にも移行し，藤野の云うように血中のα-Lipoproteinの増加としてそれが認められるようになる．そしてDissé氏腔に出現したその他の脂質の微粒子は，次いで肝実質細胞のMicrovilliのVesiculationによつて肝実質細胞内に数個宛がEncircling membrane内に囲繞されて摂取されるが，この際にも，脂質の微粒子はEncircling membrane内で互いに融合し，大きさを増して，ついにはEncircling membraneは消失し，原形質内に露呈して存在するようになる．斯くして脂質の粒子は益々融合してその大きさを増して，1μ大もの脂質滴になる．

(4) 肝実質細胞内に移行する脂質の量は注入後2～3時間で最高となり，この頃に至つて肝実質細胞内でMitochondriaの作用下にそれは酸化利用されて消失する．

(5) 教室の城谷がすでに組織顕微化学的に明らかにしたように，胸管乳糜注入時には，肝実質細胞内に出現する脂質の態度は2相性であることが，電子顕微鏡学的にも確認された．

(6) 即ち，先に教室の中村・藤野が行つた実験成績と併せ考えると，Glycerideは直接肝実質細胞内には移行し得ず，必ず一旦前述のように静脈洞内被細胞内で，Mitochondriaの作用下に酵素学的な一次的処理を受けて初めて肝実質細胞内に移行し得るのである．而も内被細胞内において，Glycerideは当然Mitochondriaの作用下に，一旦Phospholipidに変化するものであると考えなければ，この間の現象をはつきりと説明することは出来ない．